

In re Application of:
Hay and Hawkins
Application No.: 09/270,983
Filed: March 17, 1999
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PATENT
ATTY. DOCKET NO.: CIT1130-1

REMARKS

A Sequence Listing is submitted herewith. The Sequence Listing shows sequences that were present in the subject application as filed and, therefore, does not add new matter.

The specification has been amended to insert Sequence Identifiers as appropriate. As such, the amendments merely address a formality, and do not add new matter.

Attached hereto as Exhibit A is a marked copy showing the amendments to the specification. It is noted that original brackets at page 33, lines 14-15, have been maintained.

As such, the brackets in the corresponding language of Exhibit A (brackets of [25 mM Tris (pH 8.0), 50 mM NaCl, 10 mM DTT]) are not indicative of an amendment.

The Examiner is invited to contact Applicants' undersigned representative if there are any questions regarding the subject application. The Commissioner is authorized to debit (or credit) Deposit Account No. 50-1355 if any fee is required (or if there is any overpayment).

Respectfully submitted,

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EXHIBIT A
MARKED VERSION SHOWING THE AMENDMENTS TO SPECIFICATION

The paragraph at page 6, line 13, to page 7, line 2 has been amended as follows:

FIG. 1 is a schematic diagram illustrating a genetic system for monitoring caspase activity in yeast using a transcriptional reporter. Yeast were created that express a chimeric type-I transmembrane protein (CLBDG6) in which the N-terminal signal sequence and transmembrane domain (CD4) is followed by a linker consisting of 6 tetrapeptide caspase target sites (indicated in bold) that bracket the specificity of known caspases and granzyme B (Thornberry, N. A., *et al.*, *J. Biol. Chem.* 272: 17907-17911, 1997)-**DEV**DG-**WEH**DG-**IEH**DG-**IET**DG-**DEH**DG-**DQM**DG -(SEQ ID NO:[1]4) each of which is followed by a glycine residue, which acts as a stabilizing residue in the N-end rule degradation pathway in yeast (reviewed in Varshavsky, A., *Proc. Natl. Acad. Sci. USA* 93: 12142-12149, 1996). C-terminal to the caspase target site linker is a transcription factor domain, LexA-B42. The LexA-dependent transcriptional reporter consists of LexA binding sites (LexA UAS) and a promoter (P) upstream of the bacterial *lacZ* gene (*lacZ*) (FIG. 1A). The cells in FIG. 1A act as caspase activity reporters since expression of an active caspase results in CLBDG6 cleavage at the caspase target sites, releasing LexA-B42, which enters the nucleus and activates *lacZ* transcription (see FIG. 1B). A version of CLBDG6 in which the P1 aspartates are changed to glycines (CLBGG6) cannot be cleaved by caspases. Cells expressing CLBGG6 act as false positive reporters for molecules that activate *lacZ* expression independent of cleavage at caspase target site (FIG. 1C). As shown in FIG. 1D, if the cells in shown in FIG. 1B express a caspase inhibitor as well as an active caspase, caspase activity, and thus caspase-dependent release of LexA-B42, is inhibited. β -gal levels are decreased compared to cells that express the caspase alone.

The table (Table 1) at page 13, lines 1-13, to page 6, has been amended as follows:

Table 1
Characteristics of the Caspase Family

Group	Caspase	Synonym	S4-S1 recognition sequence (4 amino acids)	Substrate
<i>Group 1</i>	caspase-1	ICE	WEHD (SEQ ID NO:6), YVAD (SEQ ID NO:7)	Pro-IL1B, pro- caspases-1, -3,14
	caspase-4	ICErel-II, TX, ICH-2	(W/L)EHD (SEQ ID NO:8)	Pro-IL1B, pro- caspase-1
	caspase-5	ICErel-II, TY	(W/L)EHD (SEQ ID NO:8)	unknown
<i>Group 2</i>	caspase-3	CPP32, Yama, apopain	DEVD (SEQ ID NO:9)	PARP, DFF, SREBP, rho-GD1, pro-caspase-6, -9
	caspase-2	ICH-1		PARP
	caspase-7	Mch3, ICE- LAP3, CMH-1	DEVD (SEQ ID NO:9)	PARP, pro-caspase- 6
<i>Group 3</i>	caspase-6	Mch2	VEID (SEQ ID NO:10)	Lamins A, B1/B2, C, PARP
	caspase-8	FLICE, MAC, Mch5	LETD (SEQ ID NO:11)	PARP
	caspase-9	ICE-LAP6, Mch6	LEHD (SEQ ID NO:12)	PARP
	caspase-10	Mch4		Procaspases-3, -7

The paragraph at page 14, line 25, to page 15, line 4 has been amended as follows:

The localization sequence can be a nuclear localization sequence, an endoplasmic reticulum localization sequence, a peroxisome localization sequence, a mitochondrial localization sequence, or a localized protein. Localization sequences can be targeting sequences which are described, for example, in "Protein Targeting," Chapter 35, of Stryer, L., Biochemistry, 4th ed., W. H. Freeman, 1995. The localization sequence can also be a localized protein. Some important localization sequences include those targeting the nucleus (KKKRRK) (SEQ ID NO: 2), mitochondrion (amino terminal MLRTSSLFTRRVQPSLFRNLRRLQST-) (SEQ ID NO: 3), endoplasmic reticulum (KDEL (SEQ ID NO:5) at C-terminus, assuming a signal sequence present at N-terminus), peroxisome (SKF at C-terminus), prenylation or insertion into plasma membrane (CaaX, CC, CXC, or CCXX at C-terminus), cytoplasmic side of plasma membrane (fusion to SNAP-25), or the Golgi apparatus (fusion to furin).

The paragraph at page 33, lines 10-16 has been amended as follows:

The DIAP1 coding region was amplified by PCR using primers that generated an N-terminal myc epitope (EQKLISEEDL) (SEQ ID NO:[X]1) and introduced into the GST expression vector pGEX4T-1 (Pharmacia). The GST-myc-DIAP1 fusion protein was expressed in *E. coli* strain BL21(DE3)pLysS (Novagen) and affinity purified on glutathione-Sepharose by standard methods. The eluted protein was dialyzed against buffer A [25 mM Tris (pH 8.0), 50 mM NaCl, 10 mM DTT]. Following dialysis, the protein was frozen in aliquots after addition of glycerol to 10 %.